Remarks:

Regarding the rejection of claim 5 under 35 USC 101:

The applicant's amendments to claim 5 are believed to address and overcome the grounds of rejection.

Regarding the rejection of claim 5 under 35 USC 112, 2nd paragraph:

The applicant's amendments to claim 5 are believed to address and overcome the grounds of rejection.

Regarding the rejection of claims 1 and 4 under 35 USC 102(b) in view of Gamboa-Leon et al:

The applicant respectfully traverses the Examiner's rejection of claims 1 and 4 over the Gamboa-Leon reference, particularly in view of the currently amended claims.

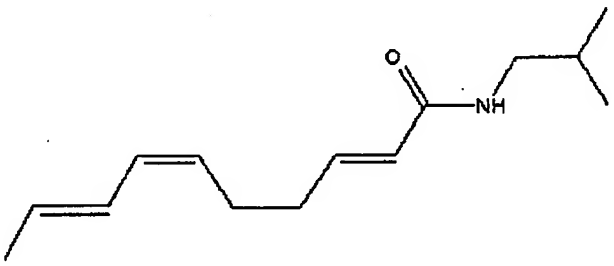
With regard to the Examiner's grounds of rejection under 35 USC §102(b), that statute holds in relevant part that a person shall be entitled to a patent unless "the invention was ... in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States." Unpatentability based on "anticipation" requires that the invention is not in fact new. See *Hoover Group, Inc. v. Custom Metalcraft, Inc.*, 66 F.3d 299, 302, 36 USPQ2d 1101, 1103 (Fed. Cir. 1995) ("lack of novelty (often called 'anticipation') requires that the same invention, including each element and limitation of the claims, was known or used by others before it was invented by the patentee"). Anticipation requires that a single reference describe the claimed invention with sufficient precision and detail to establish that the subject matter existed in the prior art. See, *In re Spada*, 911 F.2d 705, 708, 15 USPQ2d 1655, 1657 (Fed. Cir. 1990). It is the present applicants' position that this standard has not been met.

With respect now to the currently amended claims, the applicant points out that as presently amended, the compounds according to claim 1 now require that "n is 2" which introduces conjugated double bonds into the molecule adjacent to the amide group in the

molecule. The compounds provided by Gamboa-Leon do not provide any compound which meets the structural limitations which are now defined by claim 1. Accordingly, the presently amended claims are not anticipated, nor believed to be obvious over the Gamboa-Leon reference. Accordingly, reconsideration of the propriety of the outstanding rejection and its withdrawal is requested.

Regarding the rejection of claims 1,3 and 5-9 under 35 USC 102(b) and/or 103(a) in view of US 3720762 to Hatasa et al., in view of evidence from Nakatani et al..
The applicant respectfully traverses the Examiner's rejection of the claims in view of the Hatasa and Nakatani references.

With respect first to the "anticipation"-type rejection under 35 USC 102(b), the applicant points out that in light of the presently presented and amended claims it is not believed that applicant's claimed compounds are anticipated by the prior art. The Examiner's basis for the rejection under 35 USC 102(b) stems from the structure of "spilanthol", which is represented by the following:



First, with regard to the foregoing, it is visible that the compound lacks conjugated double bonds adjacent to the amide group, and would not meet the limitations of claim 1 as amended and presented in this paper.

Nor does the applicant believe that these prior art reference can be properly held as rendering the claimed invention as being obvious.

Prior to discussing the merits of the Examiner's position, the undersigned reminds the Examiner that the determination of obviousness under §103(a) requires consideration of the factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1 [148 USPQ 459] (1966): (1) the scope and content of the prior art; (2) the differences between the claims and the prior art; (3) the level of ordinary skill in the pertinent art; and (4) secondary considerations, if any, of nonobviousness. More recently in *KSR International Co. v. Teleflex Inc.*, 82 USPQ2d 1385 (U.S. 2007), the Supreme Court held that "A combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results" and, later also held "If a person of ordinary skill in the art can implement a predictable variation, and would see the benefit of doing so, §103 likely bars its patentability."

A methodology for the analysis of obviousness was set out in *In re Kotzab*, 217 F.3d 1365, 1369-70, 55 USPQ2d 1313, 1316-17 (Fed. Cir. 2000) A critical step in analyzing the patentability of claims pursuant to section 103(a) is casting the mind back to the time of invention, to consider the thinking of one of ordinary skill in the art, guided only by the prior art references and the then-accepted wisdom in the field. Close adherence to this methodology is especially important in cases where the very ease with which the invention can be understood may prompt one "to fall victim to the insidious effect of a hindsight syndrome wherein that which only the invention taught is used against its teacher."

It must also be shown that one having ordinary skill in the art would reasonably have expected any proposed changes to a prior art reference would have been successful. *Amgen, Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 1207, 18 USPQ2d 1016, 1022 (Fed. Cir. 1991); *In re O'Farrell*, 853 F.2d 894, 903-04, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988); *In re Clinton*, 527 F.2d 1226, 1228, 188 USPQ 365, 367 (CCPA 1976).

"Both the suggestion and the expectation of success must be founded in the prior art, not in the applicant's disclosure." *In re Dow Chem. Co.*, 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988).

However, an applicant may rebut a rejection based on 'obviousness' wherein it can be shown that there existed a substantial degree of unpredictability in the pertinent art area, particularly where there is evidence showing there is no reasonable expectation of similar properties in structurally similar compounds; see *In re May*, 574 F.2d 1082, 197 USPQ 601 (CCPA 1978) See also *Ex parte Blattner*, 2 USPQ2d 2047 (Bd. Pat. App. & Inter. 1987)

The "predictability or lack thereof" in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention. If one skilled in the art can readily anticipate the effect of a change within the subject matter to which the claimed invention pertains, then there is predictability in the art. On the other hand, if one skilled in the art cannot readily anticipate the effect of a change within the subject matter to which that claimed invention pertains, then there is lack of predictability in the art. Accordingly, what is known in the art provides evidence as to the question of predictability. In particular, the court in *In re Marzocchi*, 439 F.2d 220, 223-24, 169 USPQ 367, 368-70 (CCPA 1971), stated:

> "[i]n the field of chemistry generally, there may be times when the well-known unpredictability of chemical reactions will alone be enough to create a reasonable doubt as to the accuracy of a particular broad statement put forward as enabling support for a claim. This will especially be the case where the statement is, on its face, contrary to generally accepted scientific principles. Most often, additional factors, such as the teachings in pertinent references, will be available to substantiate any doubts that the asserted scope of objective enablement is in fact commensurate with the scope of protection sought and to support any demands based thereon for proof."

The applicant asserts that applicant's presently claimed invention is properly considered unobvious over the prior art references of record. The amended claim 1 is believed to define compounds which are distinguishable over the prior art of record and unobvious thereover.

Regarding the rejection of claim 2 under 35 USC 103(a) in view of Gamboa-Leon et. al., further in view of Fox et al.:

The applicants traverse the Examiner's rejection of claim 2 over the combined Gamboa-Leon and Fox references.

For the sake of brevity the applicant herein incorporates all prior remarks concerning the Gamboa-Leon references as being similarly applicable to the current grounds of rejection.

In the current Office Action, the Examiner asserts that:

17. Fox et al. teach that the substitution of an –OH for a –H of a hydrocarbon alters the boiling point and solubility of the compound.

18. The substitution of an -OH for an –H would be one that is readily apparent to one of ordinary skill in the art. As this change would alter the boiling point and water solubility of the compound, one of ordinary skill would make the substitution depending on the final use of the compound. This change would be within the abilities of one of ordinary skill, and would not require undue experimentation. The resultant compound would be expected to have a slightly altered boiling point and water solubility, and thus be more suitable for particular applications

The applicant traverses these assertions made by the Examiner as at the outset, it is well recognized in the art that flavor and fragrance chemistries are ones which are speculative in nature as even small changes in the structure of such molecules may have surprising

and unexpected (and often unwelcome) effects. This is well established in the literature, as exemplified by the following.

The applicant first makes reference to the article titled "On the Unpredictability of Odors", by C.S. Sell, as published in *Angewandte Chemie International Ed.*, 45, pp. 6254-6261 (2006) [8 sheets], by a renowned expert in the field of fragrance compounds and chemistry, Dr. C.S. Sell. A copy is enclosed for convenient review by the Examiner. Even in the introductory few paragraphs of his paper, Dr. Sell writes:

> "... Since the development of synthetic organic chemistry in the 19th century, chemists have sought a clearer understanding of the relationship between molecular structure and the odor of the molecule, largely with the intention to design novel molecules with desirable odor properties.
>
> However, this search for understanding has proved to be a very difficult task. There are many puzzling observations that often have no simple or obvious explanation. For example, on one hand, quite different molecules can have similar odors, whereas, on the other hand, similar molecules can have dissimilar odors."

Later in his same paper, at page 2 he further notes:

> "One phenomenon that seriously disrupt attempts to correlate to odor properties with molecular structure is that a given structural modification can induce a dramatic change in odor properties in one situation whilst having little or no effect in another."

Still later, near the conclusion of his paper he notes:

> "It would therefore seem that consistently accurate prediction of odors will not be possible for a very considerable time and not until a great amount of further research has been completed, the cost of which could not be borne by the flavor and fragrance industry."

The foregoing remarks of a noted authority in the field, merely echoes what is already known to skilled artisans working and that appropriate technical field, namely that the flavor and fragrance arts and their associated chemistries are highly unpredictable.

It is also well appreciated within the relevant art that the chemistries associated with the flavor and fragrance arts are known to be highly unpredictable, and that such unpredictable but substantial differences can exist even amongst chemical isomers. Evidence of such is presented the paper titled *Enantiomer separation of α-campholene and fencholene derivatives by capillary gas chromatography on permethylated cyclodextryn phases I. Compounds separable with single columns* by R. Reinhardt, et al., published in Journal of Chromatography A, 697 (1995) 475-484, a copy of which is enclosed for convenient reference. As is demonstrated by this paper, stereoisomers of a the same compound were discovered to have significantly different odor characteristics.

Now returning to the Examiner's allegations regarding the Fox reference, the applicant assert that first, there is no reason *why* a skilled artisan would have any motivation to do so, and second, that there would be no reasonable expectation of any likelihood of affecting any of the properties of the molecule. A skilled artisan reading the Fox reference would have no motivation to make any changes to these molecules extracted from plants, specifically would have no motivation to change their boiling points. As discussed above, even minor changes in the structure of compounds may have a drastic effect on the ultimate properties of such modified compounds, but such are highly unpredictable. Further, there is no indication that any change to impart a higher boiling point would have any effect whatsoever on the pungent characteristics. There is no "cause and effect" relationship known to the art. Thus, the applicant asserts that the Examiner's position in based on an impermissible "hindsight reconstruction" of the prior art which is based on a "picking-and-choosing" amongst isolated parts of the prior art wherein there is (a) no motivation to do so, and where there is (b) no reasonable likelihood of success from such changes. The Examiner is reminded that in *In re Fritch*, 972 F.2d 1260, 1266, 23 USPQ2d 1780, 1784 (Fed. Cir. 1992), the Federal Circuit stated:

> "It is impermissible to use the claimed invention as an instruction manual or "template" to piece together the teachings of the prior art so that the claimed invention is rendered obvious. *In re Gorman*, 933 F.2d 982, 987, 18 USPQ2d 1885, 1888 (Fed. Cir. 1991). This court has previously stated that "[o]ne cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." (quoting *In re Fine*, 837 F.2d at 1075, 5 USPQ2d at 1600)

See also *W.L. Gore & Associates, Inc. v. Garlock, Inc.* 220 USPQ 303 (CAFC, 1983); *In re Mercier* 185 USPQ 774, 778 (CCPA, 1975); *In re Geiger* 2 USPQ2d 1276 (CAFC, 1987).

Thus, the applicant is of the opinion that the Examiner's rejection is improper and should be withdrawn.

Regarding the rejection of claims 5 – 9 under 35 USC 103(a) in view of Gamboa-Leon in view of US 3720762 to Hatasa:

The applicant traverses the rejection of claims 5 – 9 in view of the combined Gamboa-Leon and Hatasa references.

The Examiner asserts that:

> ordinary skill would have had a reasonable expectation that the isoaffinin would have the same flavor and slight numbing properties as the spilanthol, another isobutylamide of an unsaturated fatty acid. Substitution of one compound for another would not require undue experimentation, and there would have been a reasonable expectation that the resultant foods, beverages and oral care products would maintain their favorable organoleptic properties.

The applicant traverses the Examiner's basis for the rejection and point out that first, the structures of Gamboa-Leon's N-(1-isobutyl)-2(E),4(E),8-decatrienamide is structurally

different from Hatasa's spilantol compound, and second, that as is discussed *supra*, the field of fragrance and flavor chemistries are notoriously unpredicatable. Third, the applicant directs the attention of the Examiner to claim 1 as presently presented herein, which is the "parent" claim of claims 5 – 9. It is contended then that the Examiner's rejection of claims 5 – 9 is improper and should be withdrawn.

In view of the foregoing remarks, reconsideration of the rejections raised by the Examiner is respectfully requested, and early issuance of a *Notice of Allowance* is solicited.

Should the Examiner in charge of this application believe that telephonic communication with the undersigned representative would meaningfully advance the prosecution of this application towards allowance, the Examiner is invited to contact the undersigned at their earliest convenience.

CONDITIONAL AUTHORIZATION FOR FEES

Should any further fee be required by the Commissioner in order to permit the timely entry of this paper, the Commissioner is authorized to charge any such fee to Deposit Account No. 14-1263.

Respectfully Submitted;

Andrew N. Parfomak 09 July 2008

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Structure–Odor Relationships

On the Unpredictability of Odor

*C. S. Sell**

Keywords:
fragrances · olfaction · receptors · structure–activity relationships

The relationship between molecular structure and odor has fascinated and puzzled chemists for more than a century. Despite a great deal of research on structure–odor relationships, prediction of the odor of a novel molecule remains a statistical exercise and models only provide a probability of the character, threshold, and intensity. Surprises are still commonplace, and serendipity continues to be an important factor in the discovery of novel fragrant molecules. Recent advances in our understanding of the mechanism of olfaction provide an explanation for this and suggest that our ability to predict odor properties of molecules will not improve significantly in the near future.

1. Introduction

The search to correlate the molecular structure and the odor character of a chemical compound has a long recorded history. In ancient Greece, the proponents of Democritus' atomic theory proposed that the atoms of sweet-smelling substances had smooth surfaces whereas those of acidic materials had sharp points that pricked and irritated the nose. Since the development of synthetic organic chemistry in the 19th century, chemists have sought a clearer understanding of the relationship between molecular structure and the odor of a molecule, largely with the intention to design novel molecules with desirable odor properties.

However, this search for understanding has proved to be a very difficult task. There are many puzzling observations that often have no simple or obvious explanation. For example, on one hand, quite different molecules can have similar odors, whereas, on the other hand, similar molecules can have dissimilar odors. Thus, despite the significant differences in their structures, muscone (**1**),[1] musk ketone (**2**),[2] Traseolide (**3**),[3] and Helvetolide (**4**)[4] all have similar musk odors (Figure 1), whereas the two very similar structures **5** and **6** have very different organoleptic properties: **5** has an intense urinous character while **6** is odorless.[5]

Sometimes, the functional group present in an odorant is all-important. For example, the ester group is often associated with a fruity character.[6] Thus, both Fruitate (**7**)[7] and Manzanate (**8**)[8] have distinctly fruity odors despite the differences in size and structural complexity between them (Figure 2). However, in other cases, the functional group seems unimportant: for example, structures **9**–**12** all have camphoraceous odors.[9]

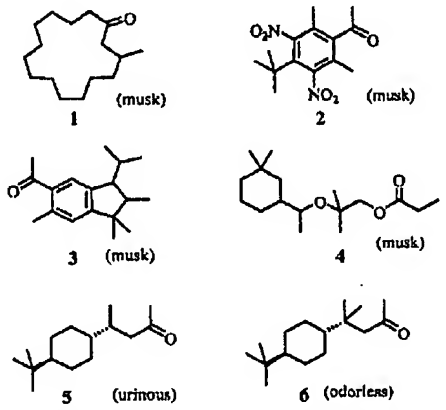

Figure 1. Different molecules, similar odors; and vice versa.

In many instances factors such as these can be brought together in triads, where the odd molecule out in structural terms is *not* the odd one out in odor. For example, of the structures **13**–**15** the last, **15**, is the odd one out in chemical terms as it is an alicyclic alcohol, whereas the other two, namely **13** and **14**, are both cinnamaldehyde derivatives (Figure 3). However, in terms of odor, it is α-methylcinna-

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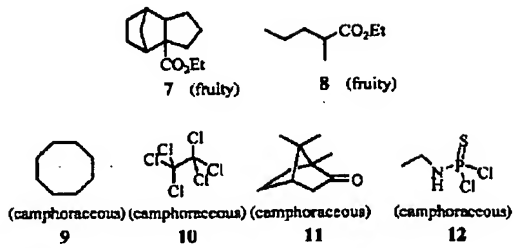


Figure 2. Role of the functional group.

maldehyde (**14**) which is the odd one out as it has a cinnamon odor[10] whereas Lilial (**13**, also known as Lily Aldehyde and Lilestralis)[11] and Florosa or Florol (**15**)[8] both have muguet (lily-of-the-valley) odors. As stated above, the ester group is usually associated with fruity scents, but *tert*-amyl acetate (**17**) has an odor which is much closer to that of camphor (**11**) than to the fruity, banana–pear scent of its isomer, *n*-amyl acetate (**16**; Figure 3).[6]

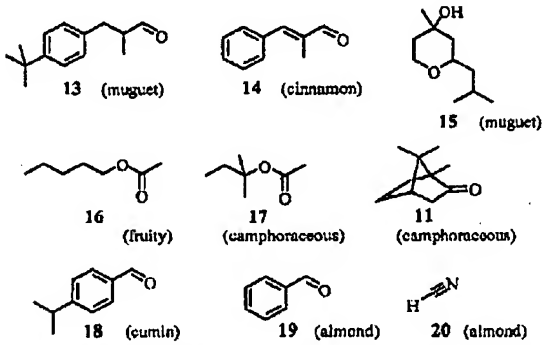
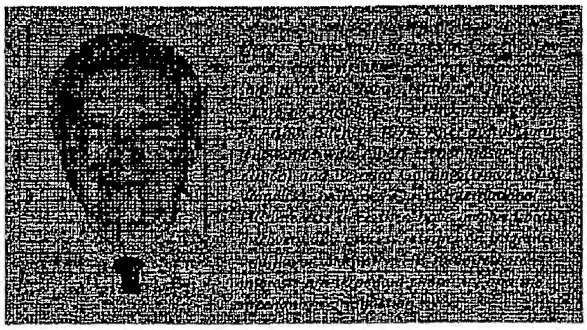


Figure 3. Triads of molecules in which the odd one out structurally is not necessarily the odd one out in odor.

Perhaps the most bizarre and best known of these triads is that involving structures **18**–**20**. Cuminaldehyde (**18**),[12] as its name suggests, smells of cumin, whereas benzaldehyde (**19**)[13] and hydrogen cyanide (**20**)[14] both smell of almond. Some theories have been proposed to account for the similarity in odor between **19** and **20**, for example, by invoking oligomerization of HCN in the receptor binding site.[15] However, the explanation is surely more likely to lie in higher-order neuroprocessing.[16]



2. Structure–Odor Models

Over the last century, many theories have been proposed relating to the primary events in olfaction and to relationships between molecular structure and odor. The review by Rossiter[17] gives an excellent summary of these theories. However, the prediction of odor remains a statistical exercise. For example, Chastrette and De Sainte Laumer developed a model based on a neural network for the prediction of the musk odor of nitrobenzene derivatives.[18] Their model gave results that were correct in 77% of test materials. Similarly, Bersuker et al. developed a model for musk activity based on an electron-topological approach.[19] Their results were impressive, yet 15 of their set of 362 materials were incorrectly predicted.

Most structure–odor models are concerned with the character of the odor. However, the commercially important parameters of detection threshold, recognition threshold, and superthreshold intensity of odorants have received much less attention, partly because of the difficulty[20] and cost of measurement of these parameters and/or because such models are even more difficult to construct. Nevertheless, detection threshold models are beginning to appear and examples include studies by Kraft on materials with marine[21] and musk[22] odor characters.

In his review of the subject, Weyerstahl concluded: "*Despite numerous excellent studies during the last 30 years the area of structure–odour relationships remains rather confusing.*"[23]

3. The Effect of Structural Modifications

One phenomenon that seriously disrupts attempts to correlate odor properties with molecular structure is that a given structural modification can induce a dramatic change in odor properties in one situation whilst having little or no effect in another. The following list of examples serves to illustrate that this is a general phenomenon that is applicable across a wide range of structural modifications and not just a few isolated instances.

The majority of odorants contain only one strongly polar function in the molecular structure. It is generally believed that this polar group will form a hydrogen bond or some other dipolar attachment to a polar site on an olfactory receptor, with the remainder of the molecule occupying a hydrophobic space in the receptor. Such polar groups have therefore come to be known as osmophoric groups or osmophores,[22] and they are used in structure–activity relationships (SARs) as a molecular reference point.[21,22] (Occasionally, a second, usually weaker, electron donor or acceptor is also involved.) Structures **21**–**24** all contain a cyclohexane ring with an osmophoric group at one end and either an isopropyl or *tert*-

butyl group at the other (Figure 4). In dihydrocryptyl acetate (**21**) and *p-tert*-butylcyclohexyl acetate (**22**), the osmophore is an acetate and there is little effect on the odor upon changing an isopropyl group for a *tert*-butyl substituent; Arctander

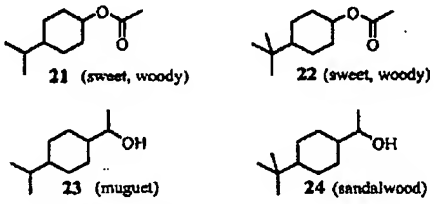


Figure 4. Role of the osmophore.

describes both as being predominantly sweet and woody in character.[24,25] However, when the osmophore is the secondary alcohol group of structures **23** and **24**, a similar small structural change has a major effect on the odor, taking it from muguet to sandalwood.[26]

Similarly, substitution of the *tert*-butyl group of **22** by an isobutyl group to give **25** changes the odor from sweet and woody to a harsh raspberry character,[27] whereas the same exchange has little effect on the muguet character of Lilial (**13**), whose isobutyl analogue Silvial (**26**) has a similar muguet odor (Figure 5).[28]

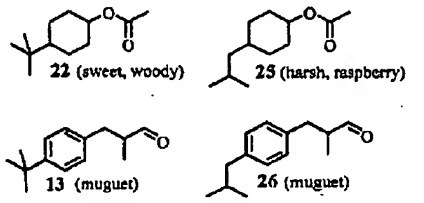


Figure 5. Role of the hydrophobic residue.

Analogous examples also exist in the case of geometric isomers. For instance, in the case of Rossitol, the odor is muguet/citrus whether the alcohol function lies *trans* (**27**) or *cis* (**28**) to the isobutyl group. However, in the analogues in which the isobutyl group has been replaced by a cyclohexyl substituent, the *trans* isomer **29** has a strong, specifically muguet odor whereas that of the *cis* isomer **30** is much weaker, woody, and only generally floral (Figure 6).[29]

The same pattern applies to geometric isomers of double bonds. (*Z*)-4-Heptenal (**31**) has a creamy, buttery odor,[30] whereas the *E* isomer **32** has an aggressive, green and putty-like odor.[31] However, in the case of 2-tetradecenal, both isomers **33** and **34** have fresh, orange odors (although that of **33** is more mandarin-like; Figure 7).[23]

Positional isomers around rings are also subject to this phenomenon. The odor of the *meta* isomer **35** of Lilial (**13**) is reported to be stronger than that of the *para* isomer,[26] whereas the odors of both *m*- and *p*-Cyclamen Aldehydes

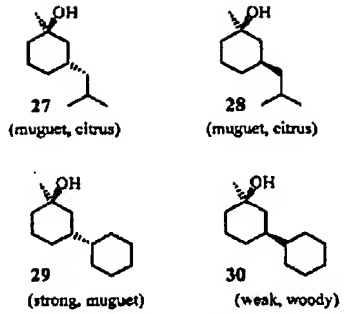


Figure 6. Odors of geometric isomers in rings.

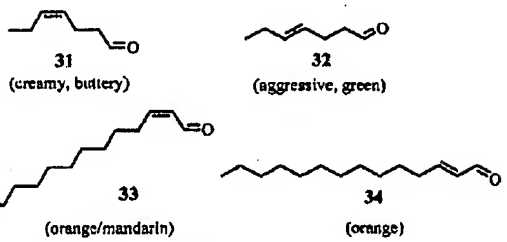


Figure 7. Odors of geometric isomers in olefins.

(**36** and **37**, respectively) are similar in character and intensity (Figure 8).[32] With the dihydrocinnamaldehydes **13** and **35–37**, the intensity of the odor varies but the character of all four remains similar. However, in the case of the acylcyclohexenes **38** and **39**, the shift from *meta* to *para* substitution results in a change in odor character from the green and fatty notes of **38** to the fruity odor of **39** (Figure 8).[33]

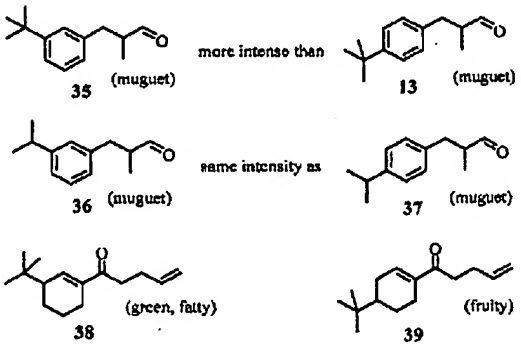


Figure 8. Effect of positional isomers on odor.

The presence or absence of a double bond also provides examples. Linalool (**40**)[34] and dihydrolinalool (**41**)[35] have similar refreshing floral, woody, and citrus odors. However, a similar saturation of a double bond with concomitant

conversion from an allylic alcohol into a saturated alcohol changes the odor from the intense, earthy mushroom character of **42**[11] to the sweet, warm, herbaceous, and nutty scent of **43** (Figure 9).[36]

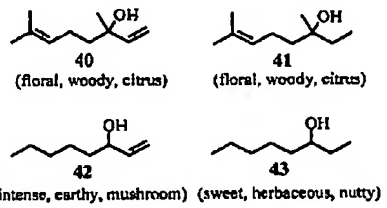


Figure 9. Effect of saturation of a double bond.

The conversion of a primary alcohol into a secondary alcohol by the addition of a methyl group also gives unpredictable results. Geraniol (**44**) is well known for its rosy odor,[37] whereas the higher homologue **45** has an intense fungal odor.[38] On the other hand, a similar transformation from Sandal Mysore Core (Santacore; **46**) to **47**, the dehydro analogue of Sandalore, has only a relatively small effect on the central sandalwood character (Figure 10).[11,39,40]

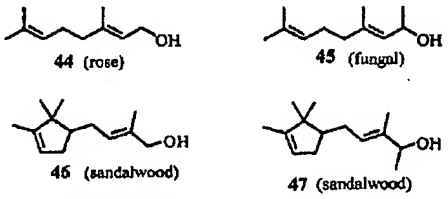


Figure 10. Effect of conversion from primary into secondary alcohols.

As stated above, functional groups are usually important in determining odor character, however, sometimes the exchange of one function for another has little or no effect. In the next three examples, a ketone group is exchanged for the acetate of the corresponding alcohol with three different levels of effect. On going from acetophenone (**48**) to styrallyl acetate (**49**), the odor shifts from sweet hawthorn-like[41] to dry, fruity, and green.[11] On moving from Patchone (**50**) to *p*-*tert*-butylcyclohexyl acetate (**51**), the degree of change is somewhat less as the odors of both materials have woody characters; that of patchone is very much on the camphoraceous and minty side of wood,[42] whereas the odor of **51** has a sweet, almost fruity character (Figure 11).[25] At the other end of this spectrum, polywood ketone (**52**) and Polywood (**53**) have similar woody, ambergris odors.[23]

Substitutions by a fragment with similar stereoelectronic properties is a common practice in fragrance ingredient discovery, just as it is in drug discovery. For example, when following up on a lead from nature, the isobutenyl group of terpenoid compounds is often substituted by a benzene ring.[43] Thus, citronellol (**54**) served as a lead for Mefrosol (also known as Phenoxanol; **55**) and the odors of both

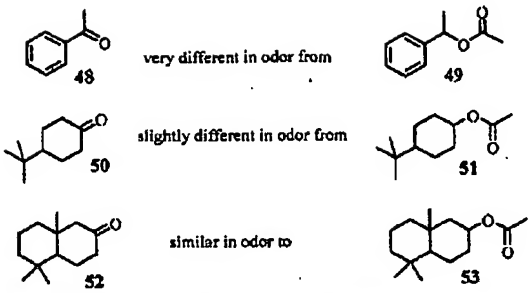


Figure 11. Effect of changing a ketone to an acetate.

compounds have a rosy character.[11] However, the green, pungent, herbaceous character of 2-methylhept-2-ene-6-one (**56**)[44] is not replicated by the sweet, floral character of benzylacetone (**57**; Figure 12).[11]

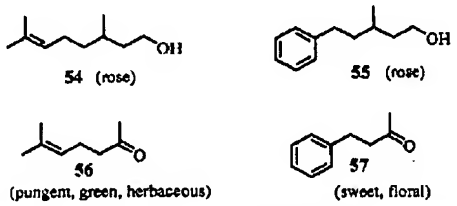


Figure 12. Effect of substitution a fragment with similar stereoelectronic properties.

Another standard substitution is that of a cyclopropane ring for a double bond. The alcohol **46** and ketone **58** both have sandalwood-like odors. Cyclopropanation of both double bonds of **46** produces Javanol (**59**), which is considered to be the strongest sandalwood-scented material known, and both "intermediate" monocyclopropanated species also have predominantly sandalwood characters (Figure 13).[45] However, cyclopropanation of one of the double bonds of **58** to give **60** leads to complete loss of the sandalwood-like odor.[33]

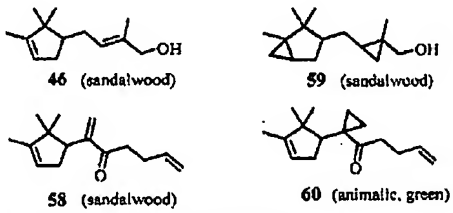
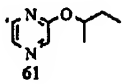


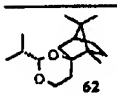
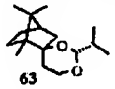
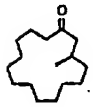
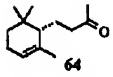
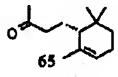
Figure 13. Effect of a cyclopropyl substituent.

Chirality also provides us with many examples of the unpredictability of the effect of changes in molecular structure on the odor of the molecule.[46] In the case of Lilial (**13**) one enantiomer is odorless,[47] whereas the odors of both


61

enantiomers of the pyrazine **61** are identical in character and threshold.[48] The pair of ethers **62** and **63** have identical thresholds of detection but different odor characters (Table 1).[49] The odors of the musks (−)-**1** and (+)-**1** have the same character, but the threshold of (−)-**1** is 0.43 ng L^{-1} whereas that of its enantiomer (+)-**1** is over twenty times higher at 9.5 ng L^{-1}.[50] The odors of the enantiomers of dihydro-α-ionone, **64** and **65**, differ in both character and threshold.[51]

Table 1: Effect of chirality on the odor of a molecule.

Compound	Odor	Threshold [ng L^{-1}]	Compound	Odor	Threshold [ng L^{-1}]
 **62**	woody, pineapple	40	 **63**	woody, rose	40
 (−)-**1**	musk	0.43	 (+)-**1**	musk	9.5
 **64**	orris	100	 **65**	violet	31

Subsequent developments based on the discoveries of Buck and Axel have enabled molecular biologists to extract the genes involved and to incorporate them into cells in culture and therefore to profile the sensitivity of individual receptor types. One interesting outcome of this discovery is that we now know that olfactory receptors are expressed in sites other than the nose and that they serve quite different functions in these other sites. An example of such work was published by Spehr et al.,[58] who showed that the human receptor hOR17-4 (which is found in sperm as well as in the nose) responds to compounds **13**, **37**, and **66–73**, but not to compounds **19** and **74–82** (Figure 14). The strongest response is observed with Bourgeonal (**70**), although this compound is not the natural substrate of the receptor in either the nose or in sperm. To the discovery chemist, this observation points towards a binding site that requires an aldehyde as a hydrogen-bond acceptor and a shape based around that of an alkyl-substituted dihydrocinnamaldehyde. This information can now be used to build a model that could be of use in designing new molecules which could potentially serve as ligands for hOR17-4. One important observation here is that the results confirm that there is not a simple correlation between receptor activity and odor. For example, phenylacetaldehyde (**66**) fires the receptor but its intense green odor[59] is a far cry from the muguet character of Lilial,[11] which is another agonist.

4. Recent Advances in Understanding Odor Perception

In the 15 years since Weyerstahl's review,[23] our ability to predict the odor of a molecule from its molecular structure has not changed much. What has changed, however, is our understanding of why such predictions are so difficult.

The major breakthrough came in 1991 when Buck and Axel identified the gene family that encodes for the olfactory receptor proteins.[52] The proteins belong to the family of seven transmembrane G-protein-coupled receptors (GPCRs) and constitute the largest family in the genome. Eight years later, Buck and co-workers[53] showed that each of the receptors was broadly tuned, in that it responds to a range of odorant molecules and that, conversely, each odorant molecule triggers a range of receptor types. This report confirmed the hypothesis proposed by Polak that the sense of smell works on a combinatorial basis.[54] Buck and Axel received the Nobel Prize in Physiology or Medicine in 2004 for their work,[55] and their accounts were published recently.[56,57] One might expect that such huge advances in our understanding of the initial stages of odor perception would help us to predict the odor of molecules, but they do not. On the contrary, the breadth of receptor tuning and the large number of receptors involved actually explain why it is so difficult and why it will remain so for a very considerable time to come.

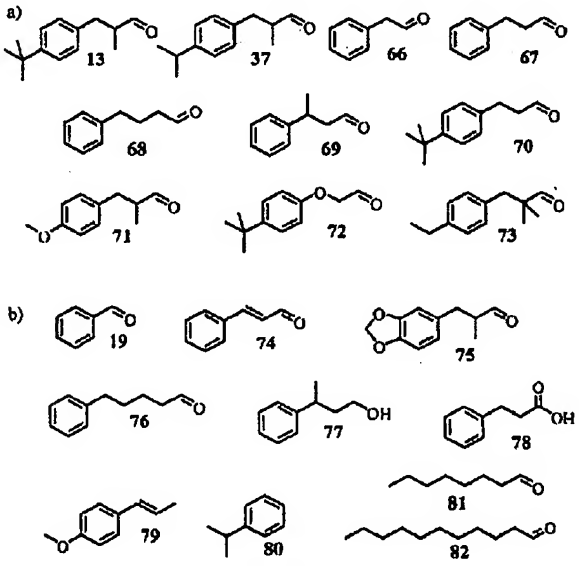


Figure 14. Agonists (a) and non-agonists (b) for hOR-17.

Araneda et al.[60] studied the receptive range of the rat olfactory receptor ORI7. Whereas Spehr et al. carried out direct measurements on the receptor cells, Araneda et al. assessed activity indirectly by measuring activity in the olfactory bulb at the glomerulus which corresponds with receptor neurons that possess ORI7. They found that this receptor responds to certain aliphatic aldehydes. From the degree of binding to 90 different test materials, they were able to establish that the binding site seems to recognize only aldehydes. There seems to be a strong steric restriction close to the aldehyde-binding point but much less strict steric requirements further away from it until a limit is reached at a chain length of 11 carbon atoms. Thus, as with hOR17-4, we can now construct a model to aid in molecular design, in this case with an aldehyde-docking area and a hydrophobic pocket with a specific overall length, and which is slightly broader at the end distant from the aldehyde.

The results described above with ORI7 and hOR17-4 point towards reasonably selective binding sites, whereas those from other receptors are much less clear-cut. For instance, Sanz et al.[61] investigated the specificity of two olfactory receptors, human class I OR52D1 and human class II OR1G1, with rather different results (Table 2). OR1G1 responds strongly to 2-ethylhexan-1-ol (**83**) and to 1-nonanol (**84**) but only weakly to 1-heptanol (**85**) and various isomeric octanols. It reveals a moderate response to decanoic acid (**86**) but no response to octanoic acid (**87**) and only a weak one to nonanoic acid (**88**). In the aldehyde series, its strongest response is to nonanal (**89**). With octanal (**81**) and decanal (**90**) it elicits only weak responses, whereas with benzaldehyde (**19**) and Lyral (**91**) it responds moderately. Of 16 esters tested, OR1G1 responds strongly only to ethyl isobutyrate (**92**); its response to the isomeric ethyl butyrate (**93**) is only weak and that to butyl butyrate (**94**) is almost zero. Strong responses were also observed to molecules as diverse as methyl thiobutanoate (**95**), benzothiazole (**96**), and γ-undecalactone (**97**). Thus OR1G1 seems to be quite selective *within* a class of substrates (for example, alcohols or acids) but not selective *between* classes, as it responds to alcohols, aldehydes, acids, esters, lactones, and a variety of heterocyclic systems. On the other hand, receptor OR52D1 generally responded more weakly to the materials in the same test set of 100 but with a different and equally puzzling pattern. It is difficult to see how one might design a model for a typical substrate for either of these receptors.

Research based on Buck and Axel's work has revealed the primary sequences of all of the human olfactory receptor proteins and now allows us to determine which are expressed in any individual. However, these proteins are proving difficult to work with experimentally and none have yet been

Table 2: Response of OR1G1 receptor to various odorant substrates.

Response			
strong	moderate	weak	none
83, **84**	**86**	**85**, **88**	**87**
89	**19**, **91**	**81**, **90**	
92, **95**, **96**, **97**		**93**	**94**

isolated in pure form. Model studies are based on extrapolation from the structure of bovine rhodopsin, which is the only GPCR for which an X-ray crystal structure exists. There are a number of assumptions in this extrapolation that must be borne in mind. Rhodopsin is an unusual GPCR in several ways, most significantly in that it requires a cofactor, 11-(*Z*)-retinal. There is also an assumption about conservation of tertiary structure from the crystalline environment to the membrane environment in which it is active. The putative binding sites in olfactory receptors are based on the binding site of 11-(*Z*)-retinal in rhodopsin rather than on in vivo experimental evidence. Spehr et al.[58] showed that activation of hOR-17 by Bourgeonal (**70**) is inhibited by undecanal (**82**), a non-agonist, and this result might suggest allosteric interactions and hence multiple binding sites. The role of odor-binding proteins (OBPs) in the olfactory mucosa is not understood and it is not known for certain whether or not they play an active role in olfaction or serve merely to remove excess odorant. However, work such as that of Spehr et al.[58] shows that, in the case of sperm or HEK cells, the receptor can be fired without the presence of an OBP.

Nonetheless, some excellent work is being done on building models of putative receptor sites and correlating these with in vivo activity. The work of Goddard and co-workers[62–64] serves as an illustration. Such work nicely complements the substrate-modeling approach of traditional SAR models.

Traditional SAR and binding-site models are essentially static in nature. A recent paper by Lai et al.[65] suggests that this might not be the best approach. Like Araneda et al.,[60] they looked at rat ORI7 receptors. Lai et al. generated a computer model of the receptor and fitted models of potential ligands into the putative binding site. They then set the whole assembly into normal motion and observed whether or not the ligand remained in the binding pocket. Molecules **81** and **98–102** remained in the binding site, whereas **103** and **104** moved out of the pocket once vibratory motion started (Figure 15). This model correlates with experiment as **81** and **98–102** are all activators in vivo whereas **103** and **104** are not. These results suggest that dynamics should be considered in addition to the traditional static stereoelectronic space-fitting approach. Prediction of new structures would be more difficult using a dynamic model and its use as an in silico screen prior to in vivo evaluation would seem more likely.

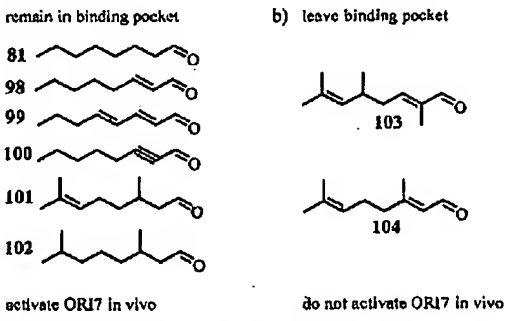


Figure 15. Results of dynamic modeling of ORI7.

5. Summary and Outlook

From all of the above examples, it is clear that even when the structure of an olfactory receptor is known it is far from certain that one could predict how well any novel potential ligand would bind to it. Facing an array of 350–400 receptors, the task would be even more difficult as a subtle change in structure might affect the binding of any one of the receptors which even a close analogue activates. In other words, the chances of correctly predicting the total pattern of receptor signaling for an odorant molecule are hundreds of times less than doing so for a pharmaceutical target. Furthermore, there are many levels of neurotransmission between the receptors and the cortex, where the signals from the receptor array are eventually interpreted as the phenomenon that we refer to as odor. At each level there are gates with opportunities for interaction between signals from different receptors and the possibility for either reduction or enhancement of individual signal components. All of this would have to be understood in order to know how the initial signal pattern would come together in the higher brain. A subtle change in signal intensity from one receptor type could have a disproportionately large effect on the overall interpretation in terms of odor character, threshold, and/or perceived intensity.

It would therefore seem that consistently accurate prediction of odors will not be possible for a very considerable time and not until a great amount of further research has been completed, the cost of which could not be borne by the flavor and fragrance industry.

In his review, Weyerstahl[23] outlined the objectives of structure–odor correlations as being 1) prediction of odors, 2) rational design of odorants, and 3) understanding the mechanism of olfaction. Since then, advances in the biosciences have greatly increased our understanding of the mechanism of olfaction but in a way that does not bode well for Weyerstahl's other two objectives. It would appear that our SAR tools will be refined and improved, although, for the foreseeable future, the prediction of odor will remain only statistical probabilities rather than certainties. There will be plenty of room for surprises and serendipity.

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JOURNAL OF CHROMATOGRAPHY A

Enantiomer separation of α-campholene and fencholene derivatives by capillary gas chromatography on permethylated cyclodextrin phases
I*. Compounds separable with single columns

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Abstract

α-Campholene and fencholene derivatives are compounds with interesting odour properties, e.g. sandalwood and woody notes. They have one (derived from the initial product, α-pinene) or several stereogenic centres. The stereoisomers of these compounds may have different odour properties; therefore, analysis methods are developed for the complete separation of the diastereomers and enantiomers. It is shown that the alcohols with one stereogenic centre as well as of the esters and ethers of α-campholene and fencholene derivatives with two stereogenic centres can be separated on permethylated α- und β-cyclodextrins dissolved in polysiloxanes. Factors influencing the separation are discussed.

1. Introduction

Apart from the characteristic functional groups, the typical odour note of a compound is determined especially by the molecular size and form [1]. Accordingly, different diastereomers as well as enantiomers may have different odour notes or even completely different types of odour [2–4].

One of the odorous substances which have been popular for more than 4000 years is the East Indian sandalwood oil. Originally, for the main substances α- and β-santalol, equal odour tones were given for all the diastereomers and enantiomers [5], but more recent studies deny this [6].

As the demand for sandalwood fragrances for a long time could not be covered by natural resources, synthetic sandal perfumes were introduced in 1940. The α-campholene [6] and fencholene compounds [7,8] found in Leipzig, which can be easily obtained from α-pineneoxide via α-campholene (1) or fencholene aldehyde (2), are suitable sandal and woody odourants. Also other substances which can be synthesized from 1 and 2, for example 3–28, have typical flowery, earthy, woody notes (e.g. 28) as well as sandal

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* For Part II, see Ref. [31].

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notes (e.g. 10 and 27). All α-campholene and fencholene compounds have one stereogenic centre corresponding to the configuration of the C-5 atom in the starting α-pinene (Fig. 1); some have several stereogenic centres due to condensation in the side chain. For the exact determination of the odour notes, also in dependence on diastereomers and enantiomers, methods are necessary for the exact differentiation of stereomers. Because all the stereomer mixtures were volatile, analysis was carried out by means of gas chromatography on chiral stationary phases.

From structurally similar classes of substances, numerous studies on furanes [9,10] are known. Likewise, lactones were analyzed on permethylated [11,12] or on different substituted alkyl derivatives [13] as well as on alkyl/acyl derivatives of the cyclodextrins [14–17]. However, the results did not permit any conclusion that the α-campholene and fencholene derivatives are separable. The first results for this class of compounds were recently presented by us [18]. In order to include also intermediate synthetics, a considerable extension of these studies was necessary. Moreover, the investigations into the connection between structure and retention for α-campholene and fencholene compounds on chiral phases fell well into line with more comprehensive studies [19] carried out on this problem.

Part I deals with the analysis of substances with one stereogenic centre. Here, mainly alcohols, but partially also compounds with other functional groups (ester, ether) are described. In Part II [31], the analysis of α-campholene and fencholene derivatives with two and more stereogenic centres is described. Often, a complete separation of all stereoisomers of such mixtures is only possible after coupling of columns.

2. Experimental

2.1. Investigated substances

The initial compounds α-campholene (1) and fencholene aldehyde (2) are obtained from α-pinene via α-pinene oxide (I): the $ZnBr_2$-catalyzed camphane rearrangement of the α-pinene oxide (Fig. 1) supplies α-campholene aldehyde (1) [7,20].

Fencholene aldehyde (2) is formed by fenchane rearrangement of the trans-pinocarveol (II), which can be obtained from α-pinene oxide (I) with aluminiumisopropylate, with subsequent hydrobromination and dehydrobromination [7,8]. By analogy, the 3-ethyl compounds (7–11) can be obtained from ethylapopinene [21]. The configuration of the used α-pinene determines the configuration of the C-1 atom in the five-membered ring of the campholene and fencholene compounds (see Fig. 1). The configura-

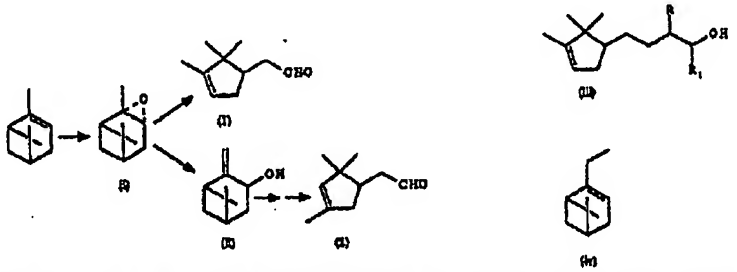

Fig. 1. Reaction scheme of the α-campholene and fencholene derivatives (1) and (2); α-pinene oxide (I); *trans*-pinocarveol (II); fragrance compounds (III) and ethylapopinene (IV).

tion of further stereogenic centres in compounds of the type III results in the course of the condensation and reduction reactions.

The individual structures of substances investigated in Part I are shown in Fig. 2.

2.2. Instrumentation

The GC–MS analyses were carried out on a Hewlett-Packard HP 5890 II gas chromatograph–HP 5971A mass-selective detector. Elec-

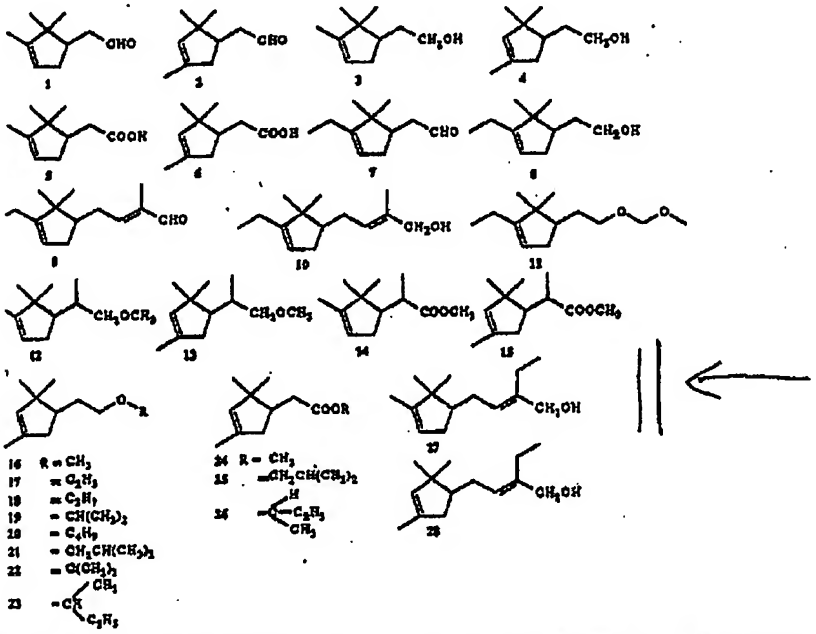

Fig. 2. Investigated substances. 1 = 2-(2,2,3-Trimethyl-3-cyclopentenyl)ethanal (α-campholene aldehyde); 2 = 2-(2,2,4-trimethyl-3-cyclopentenyl)ethanal (fencholene aldehyde); 3 = 2-(2,2,3-trimethyl-3-cyclopentenyl)ethanol; 4 = 2-(2,2,4-trimethyl-3-cyclopentenyl)ethanol; 5 = 2-(2,2,3-trimethyl-3-cyclopentenyl)acetic acid; 6 = 2-(2,2,4-trimethyl-3-cyclopentenyl)acetic acid; 7 = 2-(3-ethyl-2,2-dimethyl-3-cyclopentenyl)ethanal; 8 = 2-(3-ethyl-2,2-dimethyl-3-cyclopentenyl)ethanol; 9 = 2-methyl-4-(3-ethyl-2,2-dimethyl-3-cyclopentenyl)but-2-enal; 10 = 2-methyl-4-(3-ethyl-2,2-dimethyl-3-cyclopentenyl)but-2-enol; 11 = methoxymethyl-[2-(3-ethyl-2,2-dimethyl-3-cyclopentenyl)ethyl] ether; 12 = methyl-[2-(2,2,3-trimethyl-3-cyclopentenyl)propyl] ether; 13 = methyl-[2-(2,2,4-trimethyl-3-cyclopentenyl)propyl] ether; 14 = methyl-2-(2,2,3-trimethyl-3-cyclopentenyl)propionate; 15 = methyl-2-(2,2,4-trimethyl-3-cyclopentenyl)propionate; 16 = methyl-[2-(2,2,4-trimethyl-3-cyclopentenyl)ethyl] ether; 17 = ethyl-[2-(2,2,4-trimethyl-3-cyclopentenyl)ethyl] ether; 18 = [2-(2,2,4-trimethyl-3-cyclopentenyl)ethyl]propyl ether; 19 = isopropyl-[2-(2,2,4-trimethyl-3-cyclopentenyl)ethyl] ether; 20 = butyl-[2-(2,2,4-trimethyl-3-cyclopentenyl)ethyl] ether; 21 = 2-(2,2,4-trimethyl-3-cyclopentenyl)ethyl-(2-methylpropyl) ether; 22 = tert.-butyl-[2-(2,2,4-trimethyl-3-cyclopentenyl)ethyl] ether; 23 = but-2-yl-[2-(2,2,4-trimethyl-3-cyclopentenyl)ethyl] ether; 24 = 2-(2,2,4-trimethyl-3-cyclopentenyl)acetic acid methyl ester; 25 = 2-(2,2,4-trimethyl-3-cyclopentenyl)acetic acid (2-methylpropyl) ester; 26 = 2-(2,2,4-trimethyl-3-cyclopentenyl)acetic acid but-2-yl ester; 27 = 2-ethyl-4-(2,2,3-trimethyl-3-cyclopentenyl)but-2-enol; 28 = 2-ethyl-4-(2,2,4-trimethyl-3-cyclopentenyl)but-2-enol.

Table 1

No.	Column	Length (m)	Basic phase	Supplier
1	FS-CYCLODEX alpha I/P	50	OV-1701	CS-Chromatographie Service
2	CP-CD-β-2,3,6 M19	50	OV-1701	Chrompack
3	β-DEX 110	60	SPB 35	Supelco
4	γ-DEX 110	60	SPB 35	Supelco

tron impact ionization (70 eV) was used; the spectra were obtained in scan mode (mass range 35–400) by using helium as carrier gas. For all other analyses, a Hewlett-Packard 5890 II gas chromatograph, equipped with flame ionization detector and split/splitless injector, was available. As carrier gas, hydrogen with a split ratio of 1:100 was chosen.

The chiral resolution cR_s, given in the tables was calculated according to Eq. 1.

$$cR_s = 1.177 \cdot \frac{t_{R(2)} - t_{R(1)}}{w_{h(1)} + w_{h(2)}} \quad (1)$$

where the indices 1 and 2 refer to the first- and second-eluting enantiomers, respectively.

The capacity factors k' given in the tables were calculated from the retention times of the stereoisomers and the dead time which was estimated by coinjection of methane.

2.3. Capillary columns

The columns used are shown in Table 1. All capillaries were 0.25 mm I.D. and have the film thickness $d_f = 0.25$ μm.

3. Results and discussion

In order to distinguish the target compounds from any possible impurities and by-products, all mixtures were investigated by GC–MS on chiral and non-chiral columns. The determination of the separation and capacity factors and of the chiral resolution cR_s took place by subsequent analyses by GC–flame ionization detection (FID) at different column temperatures after optimization of the chromatographic conditions. All the substances were analyzed within a temperature interval of about 50°C. In the tables, the separation and capacity factors for the individual compounds are given only at the temperature at which the resolution was highest.

The results of the initial substances used for the separation are shown in Table 2.

Several reports are known on the separation of the two enantiomers of α-pinene [22–25] (but not the α-pinene oxide) by means of capillary GC. Permethylated α-cyclodextrin dissolved in OV-1701 shows the highest enantioselectivity (Table 3) for the simple reduction and oxidation products of α-campholene and fencholene aldehyde with only short side chains (1–6).

The separation of the non-derivatized and

Table 2
Separation of initial substances

Compound	First enantiomer	Separation factor, α	Conditions
α-Pinene	(1S)-(−)	1.050	Column 2/70°C
α-Pinene oxide	(1R)-(−)	1.091	Column 2/100°C
Ethylapopinene	R-(+)	1.018	Column 3/120°C
Ethylapopinene oxide	S-(+)	1.042	Column 3/120°C

Table 3
Separation factors (α), capacity factors (k') and chiral resolution (cR_s) on permethylated α-, β- and γ-cyclodextrins for 1–8 at column temperature T

No.	α-CD (column 1)				β-CD (column 2)				β-CD (column 3)				γ-CD (column 4)			
	α	k'	T (°C)	cR_s	α	k'	T (°C)	cR_s	α	k'	T (°C)	cR_s	α	k'	T (°C)	cR_s
1	1.025	17.31 17.75	78	1.46	1.00	17.23	80	0.00	1.006	18.62 18.74	80	0.69	1.00	10.40	90	0.00
2	1.034	13.78 14.25	78	2.80	1.00	11.39	88	0.00	1.00	9.36	90	0.00	1.00	7.65	88	0.00
3	1.021	24.87 25.39	90	1.62	1.00	32.24	90	0.00	1.00	9.57	110	0.00	1.00	10.28	110	0.00
4	1.023	21.72 22.22	88	1.41	1.008	8.99 9.07	110	0.64	1.009	7.91 7.98	110	0.82	1.00	7.61	107	0.00
5	1.030	34.18 35.21	110	1.52	1.013	48.60 49.22	110	0.96	1.013	39.60 40.13	110	1.09	1.00	25.70	120	0.00
6	1.045	20.47 21.39	115	2.24	1.059	27.86 29.51	115	2.99	1.062	16.85 17.89	120	4.63	1.016	19.08 19.38	120	1.16
7	1.00	41.78	80	0.00	1.00	21.71	110	0.00	1.00	19.40	110	0.00	*			
8	1.00	32.23	100	0.00	1.011	29.85 30.18	105	1.29	1.010	24.56 24.80	120	1.58	*			

* No values determined.

highly polar acids 5 and 6 is remarkable, especially because as a rule the analysis of carbonic acids on cyclodextrin derivatives took place after the derivatization to esters [26,27]. Only occasionally separations of non-derivatized compounds are reported [4]. Resolution is satisfactory, also on permethylated β-cyclodextrin (Fig. 3).

However, on non-polar stationary phases (OV-1), basically asymmetric peaks appear for these compounds, irrespective of their sample concentration and chromatographic conditions. The good separability on the cyclodextrin phases used should be due to the fact that the non-polar chiral selector is dissolved in medium-polar polysiloxanes.

As in the conditions for enantiomer separation, the retention time becomes very long for the carbonic acids (for the α-campholene acid 5 especially) it should be reduced by shortening the columns accordingly, as recommended earlier [28]. Therefore 5 and 6 were analyzed on a 25-m capillary with permethylated β-cyclodextrin dissolved in polysiloxane.

The results from a comparison with the 50-m capillary (Table 4) show that the chiral resolution decreases with shorter columns. If the enantioselectivity of the chiral selector is small, a separation of the enantiomers is not possible (5 on the short column). A simultaneously decrease of the column temperature to obtain a higher separation factor [29] also cannot be recommended, because the difference in retention times on the long and the short columns would be negligible small. Therefore we employ a shorter column only if the separation factor is higher than 1.04 (see also [30]).

As the synthesized compounds were obtained from (−)- or (+)-α-pinene with a great enantiomeric excess, it was possible to ascertain the elution order of the enantiomers. With the exception of 1 on column 3, the (1*R*)-enantiomers elute before the (1*S*)-enantiomers on all the columns.

The separation of the α-campholene compounds 9, 10 and 27 on permethylated cyclodextrin phases is not possible. This is remarkable because the respective fencholene compound

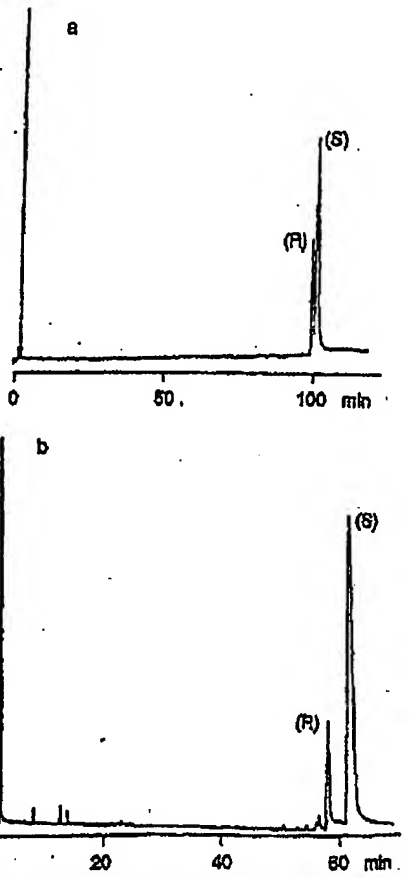

Fig. 3. Chromatograms of (a) α-campholenic acid (5) and (b) fencholenic acid (6) on permethylated β-cyclodextrin (column 2). Column temperature: 110°C (a) or 115°C (b); injection and FID temperatures: 275 and 250°C, respectively; carrier gas: hydrogen; split ratio 1:100.

(28) is separated in the same way as α-campholene derivatives with alkyl chains comparable with 9 and 10 (see [31]). Accordingly, the position of substituents on the five-membered ring and the conformative mobility as well as the

Table 4
Separation factors (α), capacity factors (k') and chiral resolution (cR_s) on capillaries with different length coated with permethylated β-cyclodextrin for α-campholane acid (5) and fencholane acid (6)

No.	50-m Capillary				25-m Capillary			
	α	k'	T (°C)	cR_s	α	k'	T (°C)	cR_s
5	1.009	19.36 19.53	125	0.78	1.00	19.70	125	0.00
	1.013	48.60 49.22	110	0.96	1.011	49.68 50.29	110	0.55
6	1.050	15.09 15.84	125	3.11	1.047	15.30 16.03	125	2.35
	1.059	27.86 29.51	115	2.99	1.059	28.74 30.45	115	2.58

distance of functional groups from the stereogenic centre in the ring have an influence on the separability of the enantiomers of α-campholane and fencholane derivatives.

The results for the ethers 12, 13 and 16–23, and esters 14, 15 and 24–26 are given in Table 5. For better comparison, ethers and esters with several stereogenic centres are not discussed in Part II but already here.

The polarity of the compounds decreases from

Table 5
Separation factors (α), capacity factors (k') and chiral resolution (cR_s) on permethylated α- and β-cyclodextrins for esters and ethers of α-campholane and fencholane derivatives at column temperature T

No.		α-CD (column 1)				β-CD (column 2)			
		α	k'	T (°C)	cR_s	α	k'	T (°C)	cR_s
12	D1[b]	1.00	29.50	70	0.00	1.00	28.64	70	0.00
	D2	1.025	32.47 33.28	70	1.29	1.00	32.57	70	0.00
13	D1	1.00	21.95	70	0.00	1.00	20.28	70	0.00
	D2	1.00	24.19	70	0.00	1.009	23.79 24.01	70	0.72
14	D1	1.00	28.31	85	0.00	1.00	26.69	85	0.00
	D2	1.018	30.60 31.15	85	1.05	1.009	29.31 29.58	85	0.74
15	D1	1.00	21.46	85	0.00	1.00	19.22	85	0.00
	D2	1.00	22.42	85	0.00	1.00	21.57	85	0.00
16		1.00	28.85	60	0.00	1.011	29.04 29.36	60	0.85
17		1.017	32.89 33.46	65	0.94	1.025	31.46 32.26	65	1.59
24		1.016	22.62 22.98	80	0.90	1.030	22.47 23.14	80	1.18
25			[a]		1.015		21.80 22.13	105	1.38
26	D1		[a]		1.022		24.07 24.61	100	2.20
	D2		[a]		1.012		24.33 24.61	100	1.09

[a] No values determined.
[b] The marking of the individual diastereomers was done schematically by D1 and D2, with D1 corresponding to the diastereomers eluating first on the polar stationary phases (Carbowax) and D2 corresponding to the diastereomer eluating last.

the corresponding alcohols via the esters to the ethers. Connected with this a decrease is found in the enantioselectivity of permethylated α- and β-cyclodextrin both for the α-campholene and the fencholene derivatives. The results show that dipole interactions between the alcohols (or the esters and ethers derived from it) and the chiral selector of the stationary phase contribute to the enantiomer differentiation on permethylated cyclodextrins but they are not their sole cause.

In comparison with **13** and **15**, the separation factors for **16** and **24** are, as expected, slightly larger both on permethylated α- and on β-cyclodextrin. Because this is also the case for α-campholene and fencholene derivatives with two stereogenic centres [31], the better separation must be due to the smaller steric hindrance of the geminal methyl groups on the five-membered ring by the α-positioned methyl substituent in the alkyl side chain.

From the methyl ether **16** to the ethyl ether **17**, the enantioselectivity increases initially on both phases, but gets completely lost with the further extension of the side chain so that **18**–**23** cannot be separated into the enantiomers. In contrast, the "chiral separation power" will change only slightly in the case of the polar esters **25** and **26**. Also the acetates of the α-campholene and fencholene alcohols **3** and **4** are excellently separated into the enantiomers.

With the increasing space requirement of the alkyl substituents, the ether oxygen of the compounds is sterically more shielded so that for interactions with cyclodextrin it is available only to a limited extent, whereas one free oxygen atom is further available for interactions in the esters. These results also are in favour of a contribution to the enantiomer differentiation from polar interactions.

In the separation of **16** and **17** as well as **24** and **25**, the *R*-enantiomers elute before the *S*-enantiomers in each case. It is remarkable that this elution sequence for **17** and **24** reverses on permethylated α-cyclodextrin (Fig. 4).

Even before, Armstrong et al. [32] and Bicchi et al. [33] had found an inversion of the elution sequence on α- and β-cyclodextrin when using similar cyclodextrin derivatives for selected

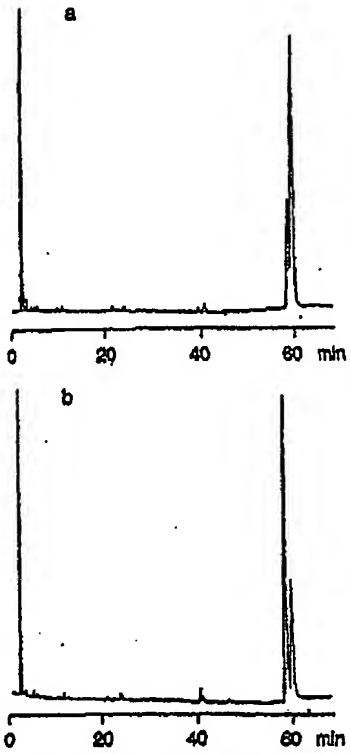

Fig. 4. Chromatograms of **17** on (a) permethylated α-cyclodextrin (column 1) and (b) permethylated β-cyclodextrin (column 2). Column temperature: 65°C; injection and FID temperature: 250°C; carrier gas: hydrogen; split ratio 1:100.

species. Consequently, by selecting the respective stationary phase, it can be made sure that an enantiomer present in excess will elute as second peak in each case. Furthermore, errors that might occur in the determination of the enantiomeric ratio or of the enantiomeric excess will be reduced in this way, for example by the peak area of a component with a small share being influenced by the tailing of the respective surplus component.

Table 6
Separation factors (α), capacity factors (k') and chiral resolution (cR_s) on permethylated β-cyclodextrin for α-campholene and fencholene derivatives with longer alkyl side chains (flavour compounds)

No.	β-CD (column 2)				β-CD (column 3)			
	α	k'	T (°C)	cR_s	α	k'	T (°C)	cR_s
27	1.00	22.19	130	0.00	1.00	21.25	130	0.00
28	1.019	15.44 16.76	130	1.69	1.016	15.60 15.85	130	1.65

α-Campholene and fencholene derivatives with woody and sandal notes (e.g. 10, 27, 28) can only be separated on permethylated β-cyclodextrin phases. Separation factors for two selected representatives are given in Table 6. In all cases, the *R*-enantiomer elutes before the *S*-enantiomer.

Different odour notes of α-campholene and fencholene compounds correlate with the different separability of the two derivatives. Obviously, the conformation differences which are due to the different position of the vinylic methyl group in the five-membered ring influence both odour notes and enantiomer separation.

4. Conclusions

Beginning with the starting substances for the synthesis of woody and sandal wood fragrances of α-campholene and fencholene types all obtained compounds can be separated very well into their enantiomers by using permethylated cyclodextrins dissolved in medium-polar polysiloxanes. For aldehydes, alcohols and acids with short alkyl side chains we employ permethylated α-cyclodextrin; for compounds with longer alkyl chains permethylated β-cyclodextrin can be recommended.

The separation is slightly dependent on the polarity of the compounds and the position of methyl substituents to each other. If there is a steric hindrance of the methyl groups on the five-membered ring, the separation will deteriorate.

Acknowledgement

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